FIG. 1

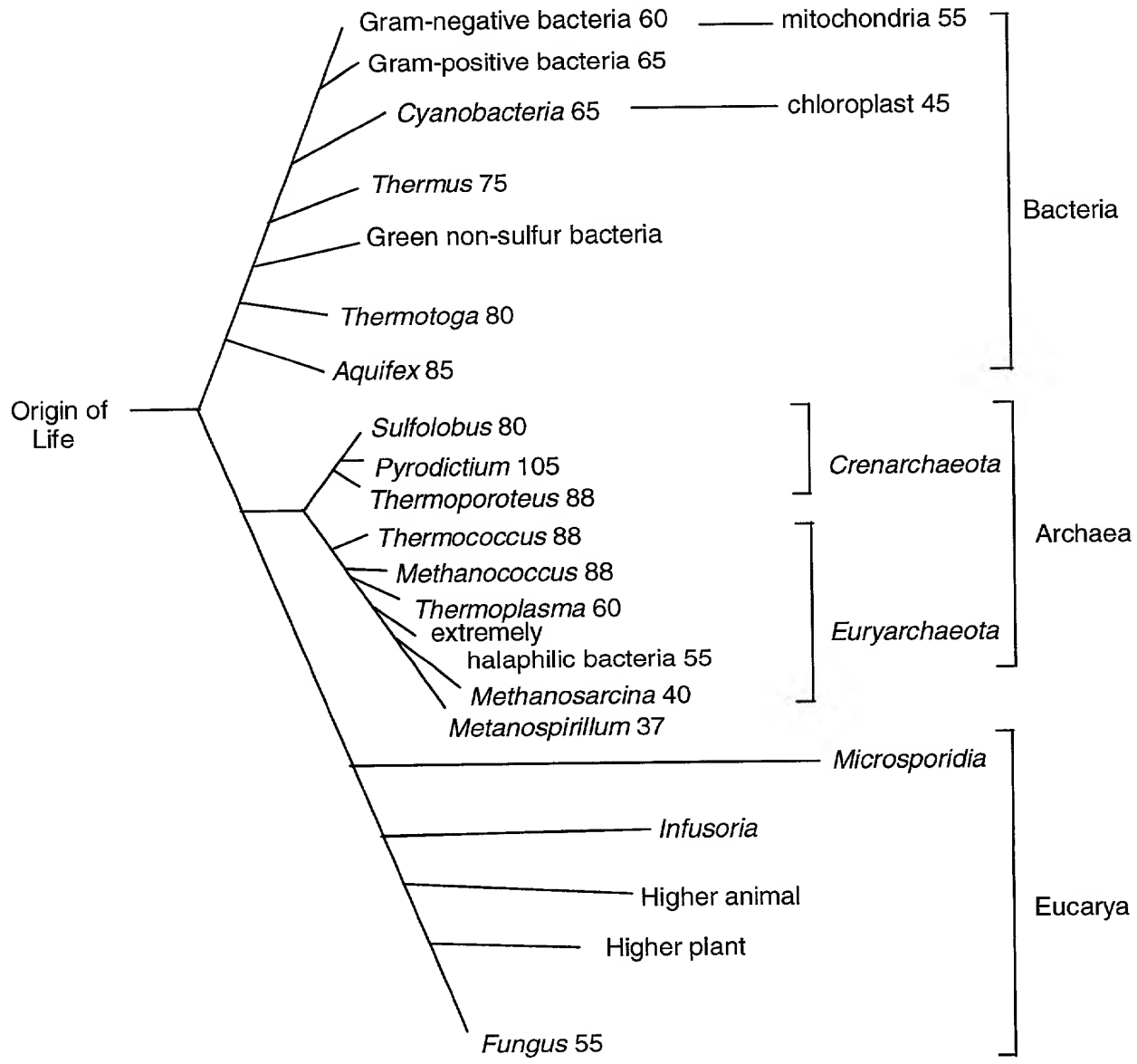

Gram-negative bacteria 60
mitochondria 55
Gram-positive bacteria 65
Cyanobacteria 65
chloroplast 45
Thermus 75
Green non-sulfur bacteria
Thermotoga 80
Aquifex 85
Bacteria
Origin of Life
Sulfolobus 80
Pyrodictium 105
Thermoporoteus 88
Crenarchaeota
Thermococcus 88
Methanococcus 88
Thermoplasma 60
extremely halaphilic bacteria 55
Methanosarcina 40
Metanospirillum 37
Euryarchaeota
Archaea
Microsporidia
Infusoria
Higher animal
Higher plant
Fungus 55
Eucarya

FIG. 2

		89 97 149 157 256 263 280 285
IPMDH	*Sulfolobus sp. strain7*	YDMYANIRP---IAKVG-LNFA---VHGAAFDI---MMYERM
	Thermus thermophilus	QDLFANLRP---VARVA-FEAA---VHGSAPDI---MMLEHA
	Bacillus subtilis	LDLFANLRP---VIREG-FKMA---VHGSAPDI---MLLRTS
	Escherichia coli	FKLFSNLRP---IARIA-FESA---AGGSAPDI---LLLRYS
	Agrobacterium tumefaciens	LELFANLRP---IASVA-FELA---VHGSAPDI---MCLRYS
	Saccharomyces cerevisiae	LQLYANLRP---ITRMAAF-MA---CHGSAPDL---MMLKLS
	Neurospora crassa	LGTYGNLRP---IARLAGF-LA---IHGSAPDI---MMLRYS
ICDH	*Saccharomyces cerevisiae*	FGLFANVRP---VIRYA-FEYA---VHGSAPDI---MMLNHM
	Bos taurus(3/4)	FDLYANVRP---IAEFA-FEYA---VHGTAPDI---MMLRHM
	Bacillus subtilis	LDLFVCLRP---LVRAA-IDYA---THGTAPKY---LLLEHL
	Escherichia coli	LDLYICLRP---LVRAA-IEYA---THGTAPKY---MMLRHM
	Ancestral residues	xDLxANLRP---IARxAxFExA---VHGSAPDI---MMLxxx

FIG. 3

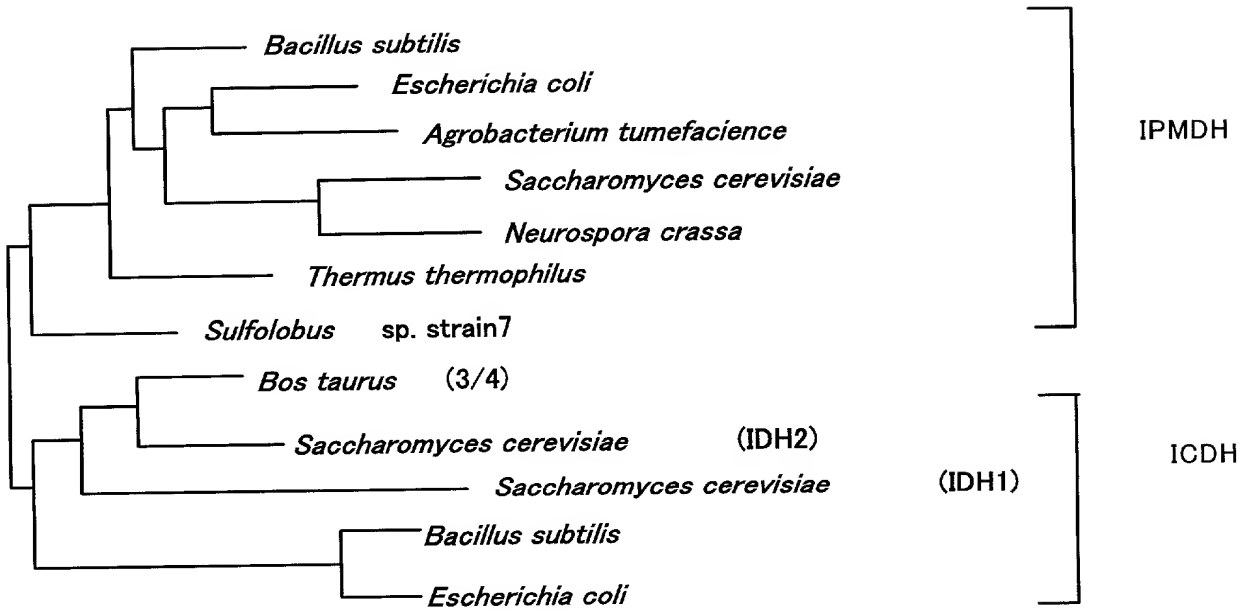
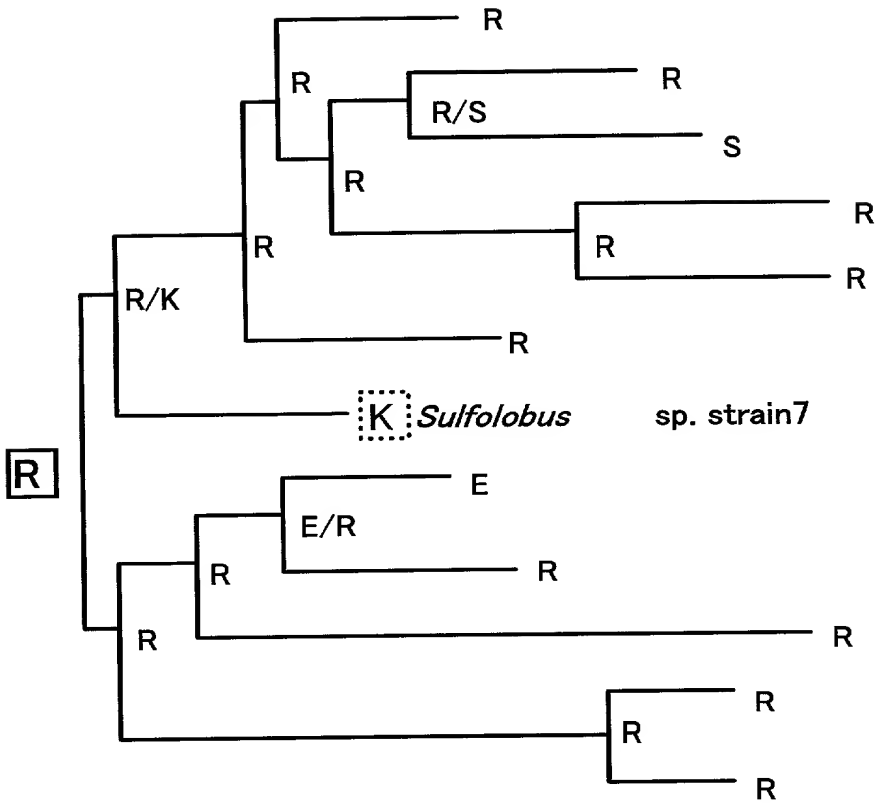

Bacillus subtilis
Escherichia coli
Agrobacterium tumefacience
Saccharomyces cerevisiae
Neurospora crassa
Thermus thermophilus
Sulfolobus sp. strain7
Bos taurus (3/4)
Saccharomyces cerevisiae (IDH2)
Saccharomyces cerevisiae (IDH1)
Bacillus subtilis
Escherichia coli
IPMDH
ICDH

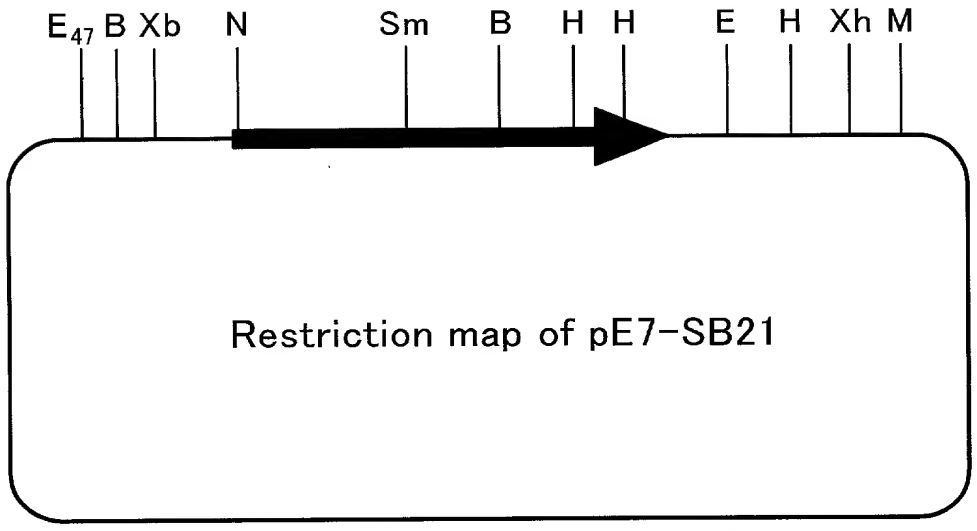
FIG. 4



IPMDH

ICDH

FIG. 5



E_{47}:*Eco*47 III, B:*Bgl* II, Xb:*Xba* I, N:*Nde* I, Sm:*Sma* I, H:*Hind* III, E;*Eco*R I, Xh:*Xho* I, M:*Mro* I

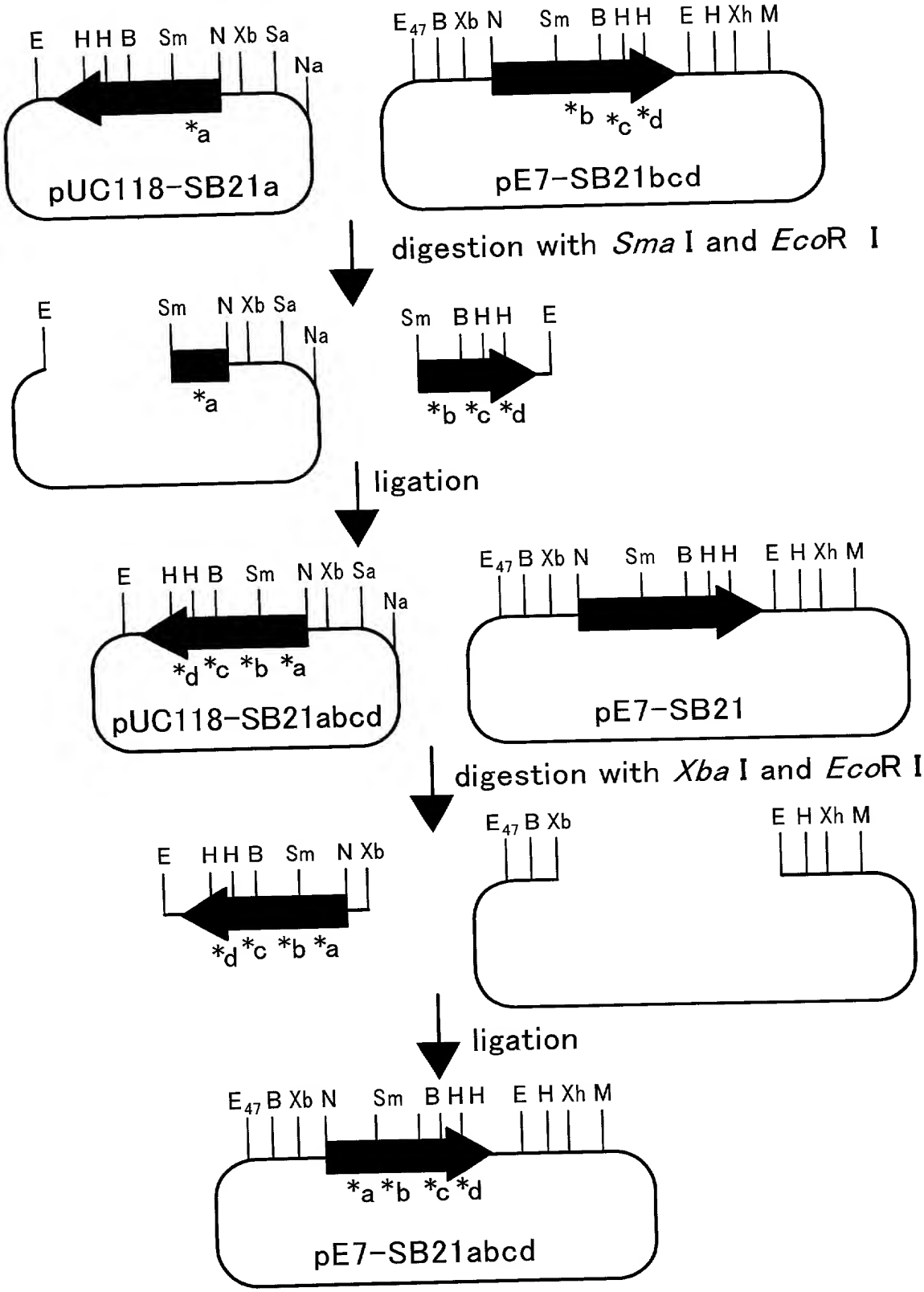
FIG. 6

```
atg ggc ttt act gtt gct tta ata caa gga gat gga att gga cca gaa   48
Met Gly Phe Thr Val Ala Leu Ile Gln Gly Asp Gly Ile Gly Pro Glu   16

ata gta tct aaa tct aag aga ata tta gcc aaa ata aat gag ctt tat   96
Ile Val Ser Lys Ser Lys Arg Ile Leu Ala Lys Ile Asn Glu Leu Tyr   32

tct ttg cct atc gaa tat att gaa gta gaa gct ggt gat cgt gca ttg  144
Ser Leu Pro Ile Glu Tyr Ile Glu Val Glu Ala Gly Asp Arg Ala Leu   48

gca aga tat ggt gaa gca ttg cca aaa gat agc tta aaa atc att gat  192
Ala Arg Tyr Gly Glu Ala Leu Pro Lys Asp Ser Leu Lys Ile Ile Asp   64

aag gcc gat ata att ttg aaa ggt cca gta gga gaa tcc gct gca gac  240
Lys Ala Asp Ile Ile Leu Lys Gly Pro Val Gly Glu Ser Ala Ala Asp   80
                    Primer P1 annealing site
gtt gtt gtc aag tta aga caa att tat gat atg tat gcc aat att aga  288
Val Val Val Lys Leu Arg Gln Ile Tyr Asp Met Tyr Ala Asn Ile Arg   96

cca gca aag tct atc ccg gga ata gat act aaa tat ggt aat gtt gat  336
Pro Ala Lys Ser Ile Pro Gly Ile Asp Thr Lys Tyr Gly Asn Val Asp  112

ata ctt ata gtg aga gaa aat act gag gat tta tac aaa ggt ttt gaa  384
Ile Leu Ile Val Arg Glu Asn Thr Glu Asp Leu Tyr Lys Gly Phe Glu  128

cat att gtt tct gat gga gta gcc gtt ggc atg aaa atc ata act aga  432
His Ile Val Ser Asp Gly Val Ala Val Gly Met Lys Ile Ile Thr Arg  144

                    Primer P4 annealing site
ttt gct tct gag aga ata gca aaa gta ggg cta aac ttt gca tta aga  480
Phe Ala Ser Glu Arg Ile Ala Lys Val Gly Leu Asn Phe Ala Leu Arg  160

agg aga aag aaa gta act tgt gtt cat aag gct aac gta atg aga att  528
Arg Arg Lys Lys Val Thr Cys Val His Lys Ala Asn Val Met Arg Ile  176

act gat ggt tta ttc gct gaa gca tgc aga tct gta tta aaa gga aaa  576
Thr Asp Gly Leu Phe Ala Glu Ala Cys Arg Ser Val Leu Lys Gly Lys  192
```

FIG. 7

```
gta gaa tat tca gaa atg tat gta gac gca gca gcg gct aat tta gta   624
Val Glu Tyr Ser Glu Met Tyr Val Asp Ala Ala Ala Ala Asn Leu Val   208

aga aat cct caa atg ttt gat gta att gta act gag aac gta tat gga   672
Arg Asn Pro Gln Met Phe Asp Val Ile Val Thr Glu Asn Val Tyr Gly   224

gac att tta agt gac gaa gct agt caa att gcg ggt agt tta ggt ata   720
Asp Ile Leu Ser Asp Glu Ala Ser Gln Ile Ala Gly Ser Leu Gly Ile   240

                                                      Primer P5
gca ccc tct gcg aat ata gga gat aaa aaa gct tta ttt gaa cca gta   768
Ala Pro Ser Ala Asn Ile Gly Asp Lys Lys Ala Leu Phe Glu Pro Val   256

anneling site
cac ggt gca gcg ttt gac att gct gga aag aat ata ggt aat ccc act   816
His Gly Ala Ala Phe Asp Ile Ala Gly Lys Asn Ile Gly Asn Pro Thr   272

                        Primer P6 annealing site
gca ttt tta ctt tct gta agt atg atg tat gaa aga atg tat gag cta   864
Ala Phe Leu Leu Ser Val Ser Met Met Tyr Glu Arg Met Tyr Glu Leu   288

tct aat gac gat aga tat ata aaa gct tca aga gct tta gaa aac gct   912
Ser Asn Asp Asp Arg Tyr Ile Lys Ala Ser Arg Ala Leu Glu Asn Ala   304

ata tac tta gtc tac aaa gag aga aaa gcg tta acc cca gat gta ggt   960
Ile Tyr Leu Val Tyr Lys Glu Arg Lys Ala Leu Thr Pro Asp Val Gly   320

ggt aat gcg aca act gat gac tta ata aat gaa att tat aat aag cta  1008
Gly Asn Ala Thr Thr Asp Asp Leu Ile Asn Glu Ile Tyr Asn Lys Leu   336

ggc taa                                                         1014
Gly
```

FIG. 8



N;*Nde* I, Sm;*Sma* I, E;*EcoR* I, E_{47};*Eco*47 III, B;*Bgl* II, Xb;*Xba* I, H;*Hind* III, Xh;*Xho* I, M;*Mro* I, Na;*Nae* I, Sa;*Sal* II,

FIG. 9

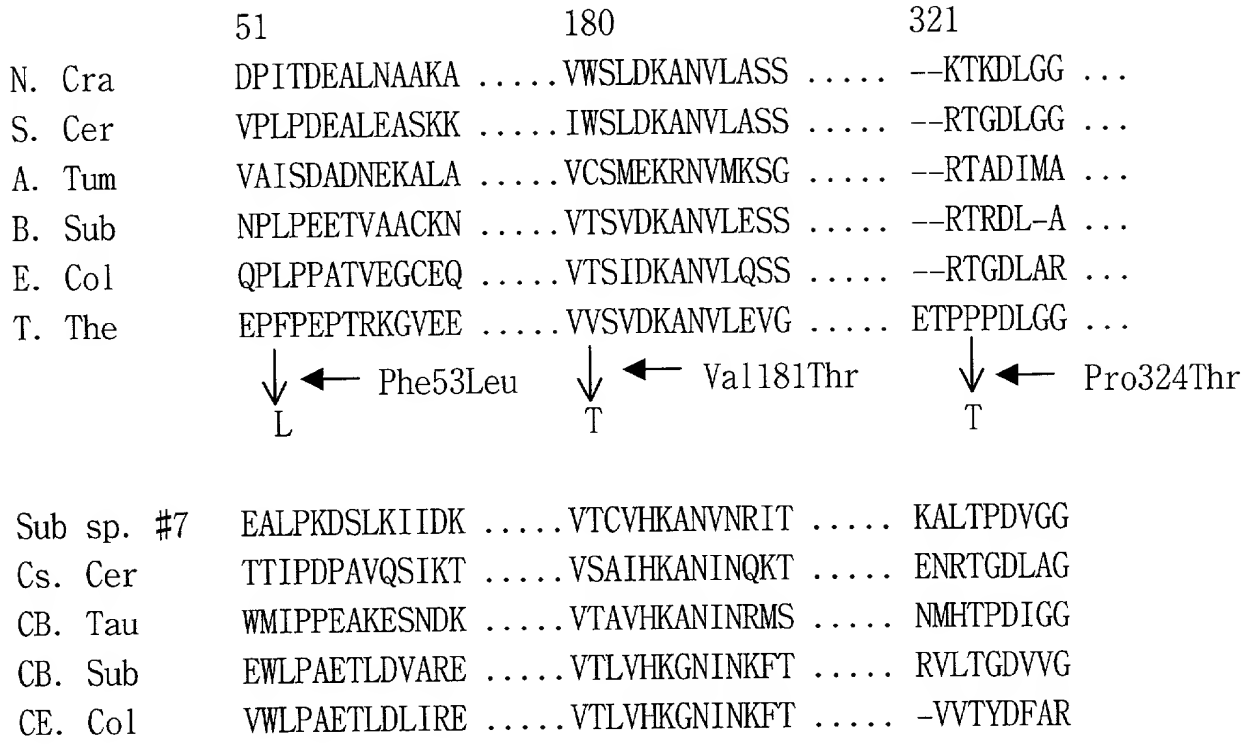

51
180
321
N. Cra DPITDEALNAAKA .....VWSLDKANVLASS ..... --KTKDLGG ...
S. Cer VPLPDEALEASKK .....IWSLDKANVLASS ..... --RTGDLGG ...
A. Tum VAISDADNEKALA .....VCSMEKRNVMKSG ..... --RTADIMA ...
B. Sub NPLPEETVAACKN .....VTSVDKANVLESS ..... --RTRDL-A ...
E. Col QPLPPATVEGCEQ .....VTSIDKANVLQSS ..... --RTGDLAR ...
T. The EPFPEPTRKGVEE .....VVSVDKANVLEVG ..... ETPPPDLGG ...
Phe53Leu
L
Val181Thr
T
Pro324Thr
T
Sub sp. #7 EALPKDSLKIIDK .....VTCVHKANVNRIT ..... KALTPDVGG
Cs. Cer TTIPDPAVQSIKT .....VSAIHKANINQKT ..... ENRTGDLAG
CB. Tau WMIPPEAKESNDK .....VTAVHKANINRMS ..... NMHTPDIGG
CB. Sub EWLPAETLDVARE .....VTLVHKGNINKFT ..... RVLTGDVVG
CE. Col VWLPAETLDLIRE .....VTLVHKGNINKFT ..... -VVTYDFAR

FIG. 10

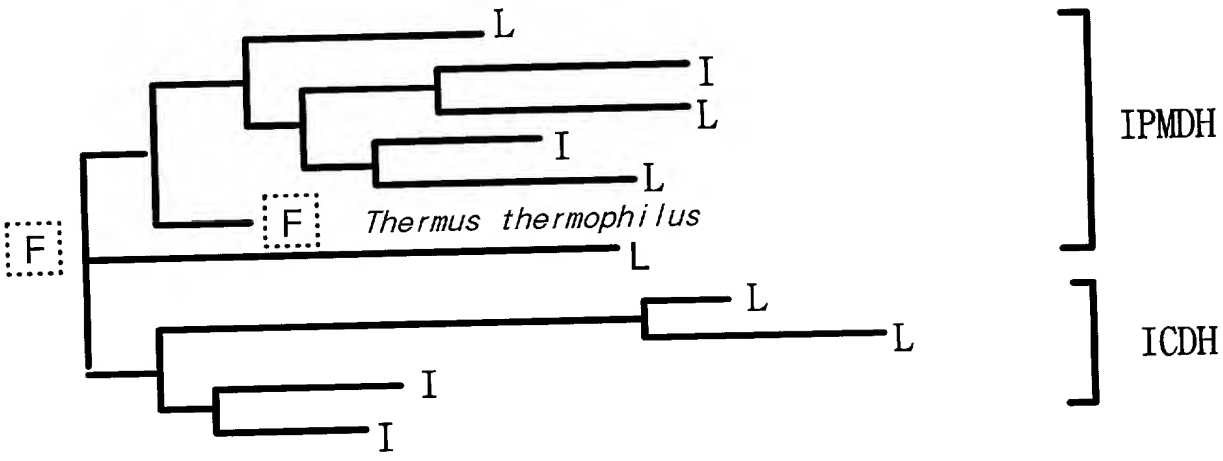


FIG. 11

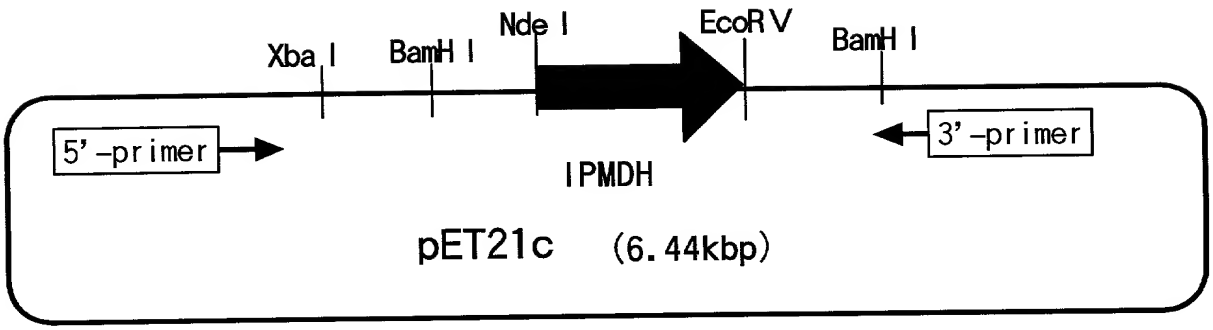


FIG. 12

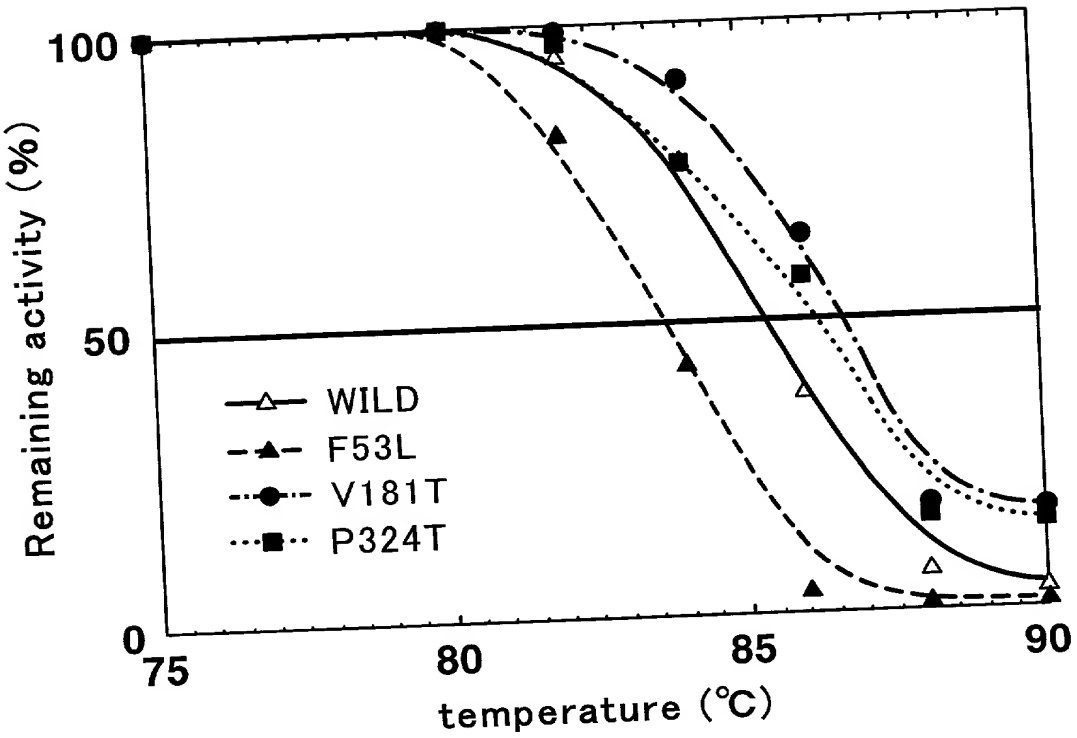
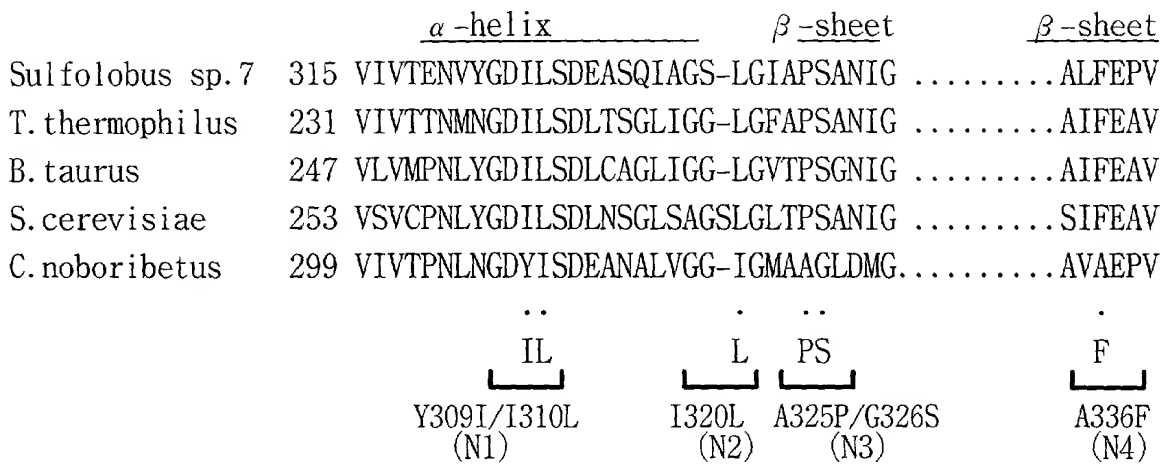

100
50
0
Remaining activity (%)
WILD
F53L
V181T
P324T
75
80
85
90
temperature (°C)

FIG. 13


α-helix
β-sheet
β-sheet
Sulfolobus sp.7 315 VIVTENVYGDILSDEASQIAGS-LGIAPSANIG .....ALFEPV
T.thermophilus 231 VIVTTNMNGDILSDLTSGLIGG-LGFAPSANIG .....AIFEAV
B.taurus 247 VLVMPNLYGDILSDLCAGLIGG-LGVTPSGNIG .....AIFEAV
S.cerevisiae 253 VSVCPNLYGDILSDLNSGLSAGSLGLTPSANIG .....SIFEAV
C.noboribetus 299 VIVTPNLNGDYISDEANALVGG-IGMAAGLDMG.....AVAEPV
IL
L
PS
F
Y309I/I310L (N1)
I320L (N2)
A325P/G326S (N3)
A336F (N4)